**Annexure 1: Standard Tissue Processing Protocol**

| S. No. | Chemicals used for Processing | Percentage | Duration |
|---|---|---|---|
| 1. | Alcohol | 50% | 1hr |
| 2. | Alcohol | 70% | 1hr |
| 3. | Alcohol | 80% | 1hr |
| 4. | Alcohol | 90% | 1hr |
| 5. | Alcohol | 100% | 1hr |
| 6. | Xylene 1 | 100% | 1.5 hr |
| 7 | Xylene 2 | 100% | 1.5 hr |

**Annexure 2: H & E Staining Protocol**

| H & E Staining Protocol | |
|---|---|
| 1. Xylene | 5 min |
| 2. Xylene | 5 min |
| 3. Xylene | 5 min |
| 4. 100% Ethanol | 1 min |
| 5. 100% Ethanol | 1 min |
| 6. 95% Ethanol | 1 min |
| 7. 95% Ethanol | 1 min |
| 8. 70% Ethanol | 1 min |
| 9. Deionized Water | 2 min |
| 10. Hematoxylin(Gill's 2x) | 2 min |
| 11. Rinse under running water | 5 minutes |
| 12. Bluing solution | 1-2 dips |
| 13. Rinse under running water | 5 minutes |
| 14. Eosin | 2-3 min |
| 15. 80% Ethanol | 3-4 dips |
| 16. 95% Ethanol | 1 min |
| 17. 95% Ethanol | 1 min |
| 18. 100% Ethanol | 1 min |
| 19. 100% Ethanol | 1 min |
| 20. Xylene I | 3 min |
| 21. Xylene II | 3 min |

**Annexure 3: Van Gieson Staining Protocol**

| **Van Gieson Staining Protocol** | |
|---|---|
| **Solution** | |
| Saturated aqueous picric acid solution | : 50 ml |
| 1% aqueous acid fuchsin solution | : 9.0 ml |
| Distilled water | : 50 ml |
| **Method** | |
| 1. Dewax section and bring to water<br>2. Stain with Weigert iron haematoxylin<br>3. Wash in tap water<br>4. Differentiate in acid alcohol<br>5. Wash in tap water<br>6. Stain with Van Gieson solution : 3 minutes<br>7. Dehydrate in various grades of alcohol<br>8. Clear in xylene and mount in DPX | |
| **Result** | |
| Nuclei | : blue/black |
| Collagen | : red |
| Other tissue | : yellow |

**Annexure 4: PAS Staining Protocol**

**Preperation of Solution**

a. Periodic Acid Solution
   Periodic Acid : 1 g
   Distilled water : 200 ml
b. Schiff's Reagent

**Method**

1. Dewax section and bring to distilled water
2. Treat with periodic acid: 5 min
3. Several changes of distilled water
4. Schiff's solution: 15 min (cover)
5. Wash: 5-10 min
6. Stain with Harris's haematoxylin
9. Differentiate in acid alcohol
10. Rinse in absolute alcohol
11. Clear in xylene and mount in DPX

**Result**

Glycogen: magenta

Nuclei: blue

**Annexure 4: DNA Extraction Protocol**

**Equipments**

1. Water bath with adjustable temperature (for incubating tissue for digestion)
2. Dry bath (optional)
3. Microtome (for cutting sections)
4. Hot plate (for heat dewaxing)
5. Refrigerated centrifuge
6. Vortex
7. Table top centrifuge
8. Pipettes (2-20 µl, 100 µl, 500-1000 µl)
9. 1.5 ml microcentrifuge tubes

**Reagents**

1. Xylene
2. Ethanol (absolute, 70%, 50%)
3. Qiagen DNA extraction kit (QiaAmp FFPE kit)

**Procedure**

**A. Dewaxing sections**

1. Cut 10-20µ thick FFPE tissue sections (4-6 sections)
2. Keep on hot plate for melting wax
3. Immerse hot slides in xylene for 5 minutes, give two changes of xylene for 5 minutes
4. Transfer slides into absolute alcohol for 3-5 minutes
5. Dip slides through 70% ethanol and 50% ethanol for 2 minutes each
6. Put the slides in coplin jar filled with water.
7. Overlap the dewaxed section over marked H&E section, scrape the marked area and put into a 1.7/2 ml microcentrifuge tube

**or**

1. Scrape tissue from slides dewax in microcentrifuge tube.
2. Add 1 mL xylenes 1.7 mL microcentrifuge tube containing tissue.
3. Vortex tube for 15-30 seconds to mix.
4. Spin in microcentrifuge for 5 minutes at full speed.
5. Pipette xylenes out of tubes into xylenes waste container- being careful not to disturb tissue pellet.
6. Repeat xylenes wash for a total of two washes.
7. Add 1mL 100% ethanol into tube.
8. Vortex tube for 15-30 seconds to mix.
9. Spin in microcentrifuge for 5 minutes at full speed.
10. Pipette ethanol out of tubes into ethanol waste container- being careful not to disturb tissue pellet.
11. Repeat 100% ethanol wash for a total of two washes.
12. Air-dry the tissue pellet. To decrease drying time, dry at 37°C–70°C.

**B**. **Tissue digestion and DNA elution**

1. Add180 µl Buffer ATL and 20 µl proteinase K in microcentrifuge tube containing tissue, vortex, centrifuge, incubate at 56°C overnight.
2. If RNA-free genomicDNA is required, add 2 µl RNaseA (100 mg/ml) and incubate for 2 minutes at room temperature.
3. Add 200 µl Buffer AL, vortex for 15-30 seconds and incubate for 10 minutes at 70°C.
4. Remove from 70°C and centrifuge briefly (5-10 seconds) to remove droplets from inside of lid.
5. Add 200µl absolute ethanol (96–100%) and mix thoroughly by vortexing (15-30 seconds)
6. Carefully transfer the entire lysate to the QIAamp Min Elute column and centrifuge at 6000 x g (8000 rpm) for 1 min, discard the filtrate from the collection tube.
7. Add 500µL of Wash buffer AW1 and centrifuge for 1 minute at 8000 rpm. Discard the filtrate, keeping the column in the same collection tube.
8. Add 500µL of Wash buffer AW2 and centrifuge for 2 minutes at 14000 rpm. Discard the filtrate and collection tube.
9. Spin column, in a clean collection tube, at 14000 rpm for additional 2 minutes. Discard filtrate and collection tube.
10. Place column in a 1.7 mL microcentrifuge. Elute the DNA by adding 50-100 µL of Buffer AE to the column and incubate at room temperature for 5 minutes. Note that eluting with 100 µL of Buffer AE is suggested when extracting DNA from small amounts of tissue.
11. Centrifuge column in tube for 1 minute at 8000 rpm. The 1.7 ml microcentrifuge tube contains the extracted DNA.
12. Store the DNA and column, if necessary, at 4°C or -20°C for further processing.
13. Quantify isolated DNA by Nanodrop (optical density 260/280).
14. Store at -4°C for immediate amplification or for longer storage at 80°C.